



Acquired Resistance to Virus Infection in Susceptible Plants

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ABSTRACT

Plants (ornamental/horticulture/agricultural) can acquire resistance to infection with viruses by appropriate treatment (inoculation) with attenuated virus strains ('vaccines'). Although action here is not analogous to that of live virus vaccines in animals, such treatments have been utilized in horticulture practice in a different way. Exocortis infection of citrus tress results in dwarfing effect that are being utilized in Australia to obtain dwarf trees by inoculation with appropriate kinds of mild strains; the dwarf trees having advantages of low cost management. The inoculation with exocortis; of course, reduces the yield in proportion to the reduction in tree size, the reduction being due to lower number of fruits per tree. But this is more than made up by the high density of planting dwarf tress (Nariani, 1979; unpublished data IOCV Sidney). Second, the most effective antiviral agents, whether of synthetic of natural origin, are the ones that act by stimulating host defense mechanism. The reduction in virus titre that follows is as high as is found in plants recovering spontaneously from systemic or necrotic infections caused by viruses. The antiviral host response apparently is inherited (Ghundron and Kassanis, 1954). Breeding of cultivars with ability to respond to antiviral agents is a possibility that should be seriously considered. Thirdly, tests with datura and potato plants infected by aphid transmitted PVY virus show that T-poly (250 ppm) can be used as an effective spray antiviral under certain circumstances (24-28 hrs before) (Khurana et al., 1978). The main lines of investigations that are of direct concern to use at the moment are the following: (a) determination of suitable host-virus combinations amenable to host-susceptibility modifying action of spray antiviral treatments; (b) method(s) of treatment of host plants for optimal expression of antiviral response; (c) determination of margin of safety of test products; (d) estimating duration of antiviral effect in treated plants, and its correlation with appearance of new protein components (Van Loon, 1975). A system of control based on use of potent antiviral agents aimed at alterations of the virus susceptibility of the host, together with a systemic insecticide plus oil in combination, may be worth the evaluation in crops or trees exposed to sever infestations by viruliferous insects.

Key words: *Acquired resistance, T-poly, Exocortis infection and Viruliferous insects.*

INTRODUCTION

Study of virus inhibition and viral interference or acquired resistance to virus disease in plants is of utmost concern and interest to plant pathologists and virologists alike because of the fact that such knowledge may have a profound bearing on the practical or theoretical considerations in the management and control of virus infection in plants. What is the state of our current thinking on the subject ? It will be the best to trace the developments in this as they have evolved and then try to speculate answers to focal questions, namely, is acquired resistance to virus in plants an expression of a form of antiviral defense? If so, what is the nature and of what use of can they be put to ?

Beginning knowledge

Apparently our knowledge of acquired tolerance or resistance to virus disease in plants is as old as our quest for knowledge of the true nature of viruses itself. Earliest observation on acquired resistance appears to be that of potato variety (yam i.e., Surinam potato or viehkartoffel), introduced in Europe from America in the mid 18th Century, that never suffered from overt virus disease but caused rapid degeneration of all other varieties grown in its vicinity (see review by Atanasoff, 1922). Brierly (1916) had earlier observed spontaneous recovery in tomato plants from mosaic disease apparently independent of external conditions. Atanasoff (1925) achieved masked carrier condition in artificially inoculated potato infected with Y-virus under field conditions. At about the same time, Johnson (1925) succeeded in transmitting viruses from 'apparently healthy' King Edward variety of potato.

Interest in study of acquired resistance to viruses in plants was not only enjoined upon until the observations made by Wingard (1928) those plants (tobacco), following a severe necrotic infection with ring spot virus (TRSV), developed young leaves 3 weeks later that looked apparently normal. Price (1932) considered this being an example of acquired immunity (Price, 1936) analogous to that obtaining in animals, the resistance being directed against only the homologous virus. If the 'apparently healthy' (recovered) scions were grafted onto healthy tobacco stock, shoots that came out showed return to ring-spot disease. These results suggested to Price that 'recovered' tobacco harbored virus in them but in low concentrations insufficient to cause disease (Price, 1936). Similar tests by Wallace (1944) conducted with scions taken from sugar beet curly top virus (SBCTV) 'recovered' tobacco plants gave conflicting results. The healthy stock of tobacco, grafted with such scions, did not develop symptoms stock unlike the tobacco/ grafted with scions taken from TRSV 'recovered' tobacco reported earlier by Price (1936). Bawden (1950) considered the stated analogy of 'acquired immunity' in vertebrates as being inapplicable in the case of plant viruses because the 'recovered' plants are not sterile.

Cross protection between viruses

The consensus developed among virologists in the thirties of last century that presence of virus in active (but mild) form is required to provide for protection against a virulent virus e.g., between related strains of TMV in tobacco (Thung, 1931; McKinney, 1929).

Viral interference tests in vivo were considered by many to be useful in determining strain relationship among viruses (McKinney, 1941; Salaman, 1933). Kohler (1943), however, found that unrelated viruses, e.g. PVX, may not be inhibited from establishing itself in the same plant independently of the first virus, such as TMV. Price (1936) observed that Turkish tobacco infected with TMV cannot be

super infected with TRSV. Also, yellow strain of TRSV would inhibit strain of the same virus in Turkish tobacco but the conserve operation did not inhibit yellow TRSV so efficiently.

The cross protection as a basis for establishing identity of viruses became a questionable procedure when Thompson (1960) obtained evidence that type strain of TMV would protect not only related aucuba strain but also apparently unrelated cabbage black ring spot virus in tobacco used as test host. Similar results were obtained by him with TMV in crude sap used as virus inoculums but not with virus in purified form. Such a finding is in agreement with that of Bawden and Price (1956) who found earlier that purified TMV gave the highest number of lesions on *N. glutinosa*, followed by the inocula from *N. tabacum*, *Phaseolus vulgaris* and *Datura stramonium*, in that order. An aspect of cross protection that attracted attention is that prior inoculation with an attenuated strain of virus (e.g. sugar beet curly top, SBCTV) protecting one susceptible variety of *Beta vulgaris* (SL 842) may not protect so well another equally susceptible variety (SL 68) under identical conditions of the test (Giddings, 1950). Similar results were obtained in experiments conducted with SBCTV in water pimpernel (*Samolus parviflorus*) by Bennet (1955), giving an indication that it is the identity of the test host that determines the outcome of results of cross protection tests. A pair of related strains of PVX was found to interfere each other well in *Datura stramonium* but not in *D. metel* under similar conditions of test (Hutton, 1948).

Interesting observations were made by Kunkel (1934) and later by Siegel (1959) in respect of cross protection between TMV strains in *N. sylvestris*. A mild strain inoculated on the lower surface from infection with the aucuba strain. The conclusion was that the immunity developing under the circumstances is not confined to 'vaccinated cells'. Evidence was that the cross establishment of a necrotic strain (lesion production) in the test plant by prior inoculation of leaves with a mottling strain was prevented very early and much before a significant increase in concentration of the protecting virus took place.

Bald (1948) observed that concentration of the protecting virus in the plant to be protected from challenge virus. Similar conclusions have been reached by Ross (1950) working with pairs of unrelated virus PVX and PVY. Ross (1959) postulated the possibility of production of distinct inhibitor molecule(s) within plant tissues as a result of virus. Direct evidence of the production or isolation of a circulating virus inhibitor inoculated plant was not forthcoming. Benda and Naylor (1958) working on *N. tabacum* plants recovering from necrotic infection (TRSV) suggested that a 'recovery factor', postulated in the circumstances, does not seem to diffuse readily out of the protected leaves.

They also found that 'recovered' tobacco plant can relapse to a diseased state if they are exposed to higher temperature (35°C) for 10 days. The relapse can be followed by recovery if test plants are again returned to cooler environments (4°C). At least one reason for relapse of symptoms due to higher temperature (28°C) is that virus (TMV) concentration is seen to go up higher in such circumstances (Bancroft and Pound, 1956).

Conflicting results obtained by Price (1936) and Wallace (1944) in the transmission of virus from infection with TRSV or SBCTV respectively could not be resolved but can now be explained if we postulate that for some as yet unknown reason the concentration of virus in tobacco recovering from SBCTV reaches a very low titre in comparison to plants recovering from TRSV. Grafting with a scion taken from SBCTV 'recovered' tobacco will therefore not give successful transmission of the disease to healthy stock plants.

In summary, our understanding of the spontaneous recovery from virus infections seen in certain species of host plants, particularly in tobacco or tomato, is that it may result from lowering of virus concentration or from production (induction), circulation and action of a non-specific virus interfering factor, both are attributable to expression of the genetic make-up of the plant and the virus strain used in inducing production. TMV local lesions interfering factor(s) are apparently present in most saps of TMV infected plants. They are heat sensitive and can be identified during virus purification procedures (Thompson, 1958). Following this observation, Loebenstein (1960 and 1962) showed that *N. glutinosa* plants can be 'immunized' against TMV by inoculating test plants with one percent TMV protein; the 'vaccine' can be given 24 hrs. Before virus, and 2 doses are better than one.

Virus interfering substances of non-viral origin

Knowledge and development of a plant virus 'vaccine' that would protect treated plants against necrotic viruses (TMV, Southern bean mosaic virus or tobacco necrotic virus) became available when it was shown that a complex polysaccharide (T-poly) obtained from fungus *Trichothecium roseum* inhibited virus infections of plants or treatment. A period of contact with leaves is necessary which determines the extent of virus inhibition. T-poly is not directly virucidal. Unlike the viral antibodies of vertebrates, T-poly does not enter into combination with viruses *in vitro*. The extent of inhibition of virus infectivity is also determined by the identity of the host plant (Gupta and Price, 1950 and 1952; Bawden and Freeman, 1952). Unlike the vaccines used for vertebrates, which is very specific for the virus, T-poly has a broad spectrum antiviral activity. Further work (Gupta et al., 1974) produced evidence that the untreated leaves of the test plants at remote site are also rendered virus (TMV) resistant, the response being dose dependent. That a circulating antiviral substance(s) may be produced by cells of treated plants at site that gets translocated to other parts of the plants was demonstrated by test of reversal of inhibition at remote site when a transcription inhibitor (actinomycin D) was applied locally at the site of treatment. These results have suggested a genetic basis for production (via DNA-dependent-RNA synthesis) of a viral interfering factor(s) inducible by T-poly.

The mechanism of antiviral action here approximates that of the interferon mechanism rather than the conventional immunological (antibody) pathway of vertebrates. Age of host plants (*N. glutinosa*) influences the expression of resistance induced by T-poly (Chandra, et al., 1978).

Antiviral agents from higher plants

Plants belonging to families such as *Amaranthaceae*, *Aizoaceae*, *Polygonaceae*, *portulacaceae* and *Rosaceae* contain powerful antiviral agents (inhibitors) in their leaf sap that inhibit transmission of viruses from these plants to plants belonging to *Scrophulariaceae*, *Solanaceae* and *leguminaceae*, possibly because they do not contain such agents. Extensive review has been published by Ragetli and Weintraub (1962 and 1974) working with carnation mosaic inhibiting factor, and also by Fantes and O'Neil (1964); with spinach leaf factor, by Kuntz and Walker (1974); with *Boerhaavia diffusa* root extract by Verma et al. (1979); with brinjal leaf extract by Verma and Mukherjee (1977). The active substances, which is most of them are glycol proteins, act via modification of host cell susceptibility as in T-poly, the complex polysaccharide ($C_{19}H_{24}O_5$) that is normally extracted in mixture with traces of double strand RNA (Gupta, 1977). Recently induction of local and systemic resistance in *N. glutinosa* against TMV by treatment with Dahlia leaf extract has been reported (Srivastava et al. 1976).

Synthetic compounds

In general four chemical types of synthetic products have been identified. *First*, pyrimidines and purine analogs, such as *thioracil* and *azaguanine* (see Mathews, 1970 for a review). These, mostly considered toxic (Singh, 1973), are thought to get incorporated into intracellular RNA, accounting in part for the inhibition of virus multiplication. *Second*, polyelectrolytes such as polyglutamic acids, are seen to act by blocking virus specific host cell receptors preventing virus entry into susceptible cells (Stahmann and Gothoskar, 1958). *Third*, double-stranded *polyribonucleotides* such as poly I: poly C, which are known to induce interferon and antiviral resistance in vertebrate cells, appear to be able to activate the antiviral resistance (virus localization) mechanism(s) of the host plant (Stein and Lobenstein, 1970). The polymer, like T-poly, has no direct virucidal action *in vitro* and it must remain in contact with treated leaves for sometime before virus challenge for the full expression of its antiviral effect. *Fourth*, *polycarboxylates* (declercq et al., 1970) which are known to induced antiviral resistance in treated *N. glutinosa*, Samsum NN tobacco or scotia beans (Stein and Loebenstein, 1972). Like poly I: poly C and T-poly, antiviral effect of the synthetic polycarboxylates is host directed and the response is dose and time dependent. As with T-poly or poly I: poly C, the development of antiviral resistance to polycarboxylates in test plants can be reversed by judicious use of actinomycin D. The authors also noticed that the inducible antiviral resistance seems to vary with species of host. Similar observations have been made by Gianinazzi and Kassains (1974) working with resistance induced by polyacrylic acid.

Oil sprays

About two dozen viruses for which inhibition (up to 83%) of aphid transmission by mineral oil emulsions under field condition is possible have been reported (Vanderveken, 1977).

Control of aphid transmission of viruses have been more effective with stylet borne viruses rather than with persistent type (circulative) viruses. The mechanism by which oil sprayings render plants resistant to virus spread is not fully known but most likely is achieved by altering the virus vector relationship. Inhibitory effects on the aphid transmission of non persistent (Bradley et al., 1962; Bhargava and Khurana, 1969).

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